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Atrial stretch delays gastric emptying of liquids in awake rats

R.C. Palheta Jr. [a,b], M.T.B. Silva [a], H.L.G. Barbosa [a], A.D.N. Pinheiro [a], K.V.V. Cardoso [a], J.R.V. Graça [a], P.J.C. Magalhães [a], R.B. Oliveira [c], A.A. Santos [a,*]

[a] Department of Physiology and Pharmacology, School of Medicine, Federal University of Ceará, Brazil
[b] School of Veterinary Medicine, Federal University of Vale do São Francisco, Brazil
[c] Department of Medical Clinics, Ribeirão Preto School of Medicine, São Paulo University, Brazil

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ABSTRACT

Aims: We previously reported that mechanical atrial stretch (AS) by balloon distention increased gastric tonus in anesthetized rats. The present study evaluated the effect of AS on the gastric emptying of a liquid test meal in awake rats and its underlying neural mechanisms.
Main methods: Anesthetized male rats received a balloon catheter into the right atrium and a gastrostomy cannula. The next day, mean arterial pressure (MAP), heart rate (HR), central venous pressure (CVP), and cardiac output (CO) were continuously monitored. After the first 20 min of monitoring (basal interval), the balloon was either distended or not (control) with 30, 50, or 70 μl saline for 5 min. Fifteen minutes later, the rats received the test meal (glucose solution with phenol red), and fractional gastric dye retention was determined 10, 20, or 30 min later.
Key findings: Heart rate and CVP values were transiently increased by 50 or 70 μl AS but not 30 μl AS, whereas gastric emptying was slower after 30, 50, or 70 μl AS than after sham distention. Subdiaphragmatic vagotomy or splanchnicotomy + celiac ganglionectomy and capsaicin, ondansetron, hexamethonium, L-NAME, and glibenclamide treatment prevented the AS-induced delay in gastric emptying, whereas atropine and guanethidine treatment failed to prevent it.
Significance: Atrial stretch inhibited the gastric emptying of liquid via non-adrenergic and non-cholinergic pathways that activate nitric oxide-K^+_{ATP} channels.

Introduction

Capelo et al. (1983) reported that hypervolemia caused by the infusion of isotonic saline enhanced gastric tonus in anesthetized dogs, whereas hypovolemia induced by bleeding decreased gastric tonus. However, the precise role of blood volume in such a phenomenon is uncertain because saline infusion also induces hemodilution, acidosis, and hypoxemia, which might modulate gut motility (Tack, 2006). Blood transfusion in rats increases gastric tonus for at least 60 min, whereas acute bleeding reduces gastric tonus that is reversed by blood replacement (Graça et al., 2002).

Blood volume homeostasis is achieved via a complex process that involves afferent pathways triggered by low-pressure mechanoreceptors located in several venous territories, including the cardiac atria (Antunes-Rodrigues et al., 2004; Michell et al., 2008). We found that mechanical distention of the right atrium using a balloon catheter in anesthetized rats volume-dependently increased gastric tonus (Palheta et al., 2010). Gastric tonus is the major driving factor for the gastric emptying (GE) of liquid meals (Tack, 2006), and we hypothesized that atrial stretch (AS) also alters GE. The present study investigated the following: (*i*) the effect of mechanical stretch of the right atrium on GE and the small intestinal transit of a liquid test meal in awake rats and (*ii*) the neural pathways that underlie such effects.

Materials and methods

Animals and surgical procedures

Animal handling followed the International Guiding Principles for Research Involving Animals (International Council for Laboratory Animal Science) after approval from the Ethics Committee on Animal Experiments of the Federal University of Ceará (CEPA #02/2009). Male Wistar rats (250–280 g, $n = 266$) were obtained from the central housing station of the Federal University of Ceará and maintained in a temperature-controlled room on a 12 h/12 h light/dark cycle. The animals were then isolated in Bollman's cages and fasted for 18 h with free access to an oral rehydration solution (75 mM Na^+, 65 mM Cl^-, 20 mM K^+, 75 mM glucose, and 10 mM citrate). This procedure ensures clearing the stomach of residues while maintaining euglycemia and normovolemia (Souza et al., 2009).

* Corresponding author at: Laboratório Escola Prof. Luiz Capelo, Departamento de Fisiologia e Farmacologia, Faculdade de Medicina, Universidade Federal do Ceará, Caixa Postal 3157, CEP 60.430-270 Fortaleza, Brazil. Fax: +55 85 3366 8333.
E-mail address: meno@ufc.br (A.A. Santos).

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Under ether anesthesia, the rats underwent laparotomy, and a midgastric incision was made for the insertion of a plastic tube (6 mm outer diameter) whose distal end was positioned at the gastric fundus or at the first 1.0 cm of the duodenal bulb, respectively, referred to as the *gastric cannula* or *duodenal cannula*. They were fixed at the gastric wall, and the proximal end was externalized at the interscapular region. A polyethylene cannula (PE-50, Intramedic Clay Adams, Franklin Lakes, NJ, USA) with a thermocouple sensor was inserted into the carotid artery to determine cardiac output (CO; in ml min^{-1}) by thermodilution (Palheta et al., 2010). The right jugular vein received two catheters united by cyanoacrylate glue. The first one consisted of a balloon catheter (0.51 mm inner diameter, 0.94 mm outer diameter; Silastic, Dow Corning, Midland, MI, USA), manufactured as described previously (Palheta et al., 2010). The length of the second catheter (PE-10) was 1.0 cm shorter than the former. The balloon catheter was passed into the right jugular vein and advanced down to the superior vena cava so that its tip laid at the right atrium to stretch it (Palheta et al., 2010). Once externalized, the vascular catheters were connected to pressure transducers coupled to a digital acquisition system (PowerLab/8SP, AD Instruments, Bella Vista, NSW, Australia) for the continuous recording of mean arterial pressure (MAP; in mmHg) and central venous pressure (CVP; in cmH_2O). This technique allowed simultaneous mechanical stretching of the right atrium and CVP monitoring. Three stainless steel wires (0.203 mm Teflon-coated; A.M. Systems, Carlsborg, WA, USA) were fixed bilaterally onto the chest muscles and hip muscle of the left paw, externalized at the interscapular region. After connecting them to a bioamplifier (ML132 BioAmp) coupled to the digital system, an electrocardiogram signal was derived to continuously record heart rate (HR; in beats per minute [bpm]). A 72 h interval was allowed for recovery after surgery.

Experimental design for upper gastrointestinal transit assessment

Gastric emptying of liquid test meal

To test the effect of AS on GE, the rats were continuously monitored for up to 70 min. During the initial 20 min (termed the basal interval; Fig. 2), the atrial balloon was left untouched. The rats were then randomly assigned to either the control or AS protocol as previously reported (Palheta et al., 2010). For the rats subjected to AS, the balloon was distended once for 5 min (i.e., 20–25 min interval; Fig. 2; *AS*) with 50 μl saline. For the rats in the control group, the balloon remained empty during the 20–25 min interval (Fig. 2; *Sham*, Sh) as well as up to the end of the study. After balloon deflation or the sham-procedure, the rats were given a 5 min rest interval (Fig. 2, *R*). Ten minutes later, the rats received 1.5 ml of a test meal (0.5 mg ml^{-1} of phenol red in 5% glucose solution; Fig. 2, *Gavage*) via the gastric cannula. After a 10, 20, or 30 min postprandial interval, the animals were sacrificed (Fig. 2, *PP-10*, *PP-20*, and *PP-30*, respectively) by an overdose of thiopental for the determination of fractional gastric dye recovery as previously described (Souza et al., 2009). The other rats were subjected to AS for 5 min with 30 or 70 μl after the basal interval. Fifteen minutes later, the rats received the test meal and were sacrificed 10 min postprandially for the determination of gastric dye recovery as described below.

After laparotomy, the gut was divided into consecutive segments: the stomach and small intestine. The volume of each individual segment was calculated by submerging it in a graduated cylinder that contained 100 ml of 0.1 N NaOH. After segment homogenization, the proteins were precipitated with 0.5 ml of 20% trichloroacetic acid. After centrifugation, 3 ml of the supernatant was added to 4 ml of 0.5 N NaOH, and the samples were read by a spectrophotometer at 560 nm to construct dilution curves by plotting the dye concentrations against optical densities. The value of fractional (%) gastric dye recovery is expressed according to the following equation:

$$\text{Gastric dye retention}(\%) = 1 - \left[\frac{\text{amount of phenol red recovered in stomach}}{\text{total amount of phenol red recovered from all segments}}\right] \times 100$$

Surgical and pharmacological protocols

To determine the effect of hypovolemia on the present phenomenon, a separate group of rats was pretreated (4 h) subcutaneously with 5 ml of polyethylene glycol (PEG; 20 M carbowax; Union Carbide; 30% w/w; Stricker and MacArthur, 1974). After blood sample collection for hematocrit analysis, the animals were randomly subjected to control or 50 μl AS protocols, followed by test meal administration and sacrifice 10 min later for GE assessment as described above.

To verify the role of cardiac afferent C-fibers on the present phenomenon, another set of rats was anesthetized and treated with capsaicin (0.1 ml, 1 mg ml^{-1}) according to Kaufman and Deng (2004). After 15 min, they received a second dose of capsaicin, also instilled into the pericardial space, which was rinsed 30 min later with isotonic saline (5 ml), followed by suture of the incision. The respective controls were subjected to the same procedure, with the exception that they were instilled with vehicle (10% Tween) instead of capsaicin. Three days later, both capsaicin- and vehicle-treated rats were subjected to 50 μl AS, fed the test meal, and sacrificed 10 min later for GE assessment as described above.

To evaluate the neural pathways involved in the present phenomenon, other rats were subjected to bilateral subdiaphragmatic vagotomy via circular seromuscular myotomy of the esophagus 2 cm from the gastroesophageal junction (Hansen and Krueger, 1997). In another group, the rats were subjected to celiac ganglionectomy and splanchnic nerve sectioning (Fujita and Donovan, 2005). Three days later, the denervated and respective sham-operated rats were subjected to the 50 μl AS protocol, fed the test meal, and sacrificed 10 min later for GE assessment as described above.

To assess neurotransmission involved in the present phenomenon, other rats received an intravenous (1 ml·kg^{-1}) injection of one of the following agents: saline (control), hexamethonium (10 mg·kg^{-1}), atropine (0.5 mg·kg^{-1}), guanethidine sulfate (10 mg·kg^{-1}), or ondansetron (20 μg·kg^{-1}). The nitrergic contribution was assessed by pretreatment with Nω-nitro-L-arginine methyl ester (L-NAME; 3 mg·kg^{-1}), L-arginine (100 mg·kg^{-1}) + L-NAME (3 mg·kg^{-1}), or methylene blue (3 mg·kg^{-1}). The role of K^+-ATP channels was assessed by pretreatment with glibenclamide (1 mg·kg^{-1}) either alone or combined with diazoxide (3 mg·kg^{-1}). All of the doses were selected based on previous studies (Gondim et al., 1999; Medeiros et al., 2008). After 10 or 60 min (guanethidine subset) pharmacological pretreatment, the rats were randomly subjected to the control or 50 μl AS protocol, fed the test meal, and sacrificed 10 min later for gut motility assessment as described above.

Assessment of small intestinal transit

A separate set of rats was subjected to either 50 μl AS or the sham protocol and received 1 ml of the liquid test meal 15 min later injected directly into the duodenum. After 20 min, the rats were sacrificed to determine fractional dye recovery. After laparotomy, the stomach and first 1 cm segment of the duodenum (with the cannula tip) were removed, comprising segment 1. The remaining gut was carefully removed and stretched. Obstructive ligatures were performed to obtain five consecutive small intestine segments (~20 cm long). Each segment was homogenized, and the dye content was determined by spectrophotometry as described above. Fractional marker recovery was calculated for each gut segment as the ratio